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Docket No.: 02650/100F966-US2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Richard D. Granstein

Confirmation No. 8709

Application No.: 09/679,776

Art Unit: 1632

Filed: October 5, 2000

Examiner: Q. Li

For: PROTECTIVE IMMUNITY OR IMMUNOLOGICAL TOLERANCE INDUCED WITH RNA, PARTICULARLY TOTAL CELLULAR RNA

DECLARATION OF RICHARD D. GRANSTEIN UNDER 37 C.F.R. § 1.132

MS Non-Fee Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Richard D. Granstein, declare that:

1. I am a citizen of the United States and reside in Greenburgh, New York.

2. I received a M.D. from the University of California School of Medicine in 1978.

3. I am currently the Chairman of Dermatology at Weil Medical College of Cornell University, New York (the Assignee of the above-identified U.S. Patent Application) where I have been employed since 1995. I specialize in dermatology and am particularly interested in immunologic disorders of the skin. My curriculum vitae is attached as Exhibit A.

4. I am the named inventor of the above-identified U.S. Patent Application.

5. I have read the Office Action dated July 29, 2003 and understand that claims 16-19, and 21-23 relating to inducing immune tolerance to an antigen, stand rejected by the Examiner. I further understand that the Examiner believes that it is "not appropriate to use the response to tumor cells as the sole support for microbial, allergen, autoantigen or transplantation antigen".

6. The following experiments involving intravenous administration of total cellular RNA from spleens of mice of the same strain as skin donors were performed to determine the response to skin grafts, and hence, transplant tissue antigens. These experiments were performed by me or under my supervision, and demonstrate that the claimed invention (particularly new claims 43-49) dramatically increases tolerance to transplant tissue antigens.

A. A 1x1 cm area of skin was excised from 15 donor mice. Before excising the skin graft, dorsal hair was clipped and sodium thioglycolate (Neet, Whitehall Laboratories) was applied to the clipped site. The sodium thiogylcolate was washed off with tap water and the cutaneous surface was cleaned twice with 70% isopropyl alcohol.

B. The panniculus carnosus and subcutaneous tissue were mechanically removed from the skin grafts. They were then floated dermis side down on sterile phosphate buffered saline (PBS). All procedures performed in the preparation of these skin grafts were performed using sterile techniques in a laminar flow hood.

C. Total cellular RNA was prepared from spleens of mice of the same strain as the skin donors. 50 μg of total cellular RNA (BALB/c RNA)

and phosphate buffered saline (PBS) was administered intravenously to five recipient mice six and seven days prior to skin grafting.

D. A group of five control mice were intravenously injected with PBS alone six and seven days prior to skin grafting.

E. Recipient and control mice were anesthetized. A 0.75 x 0.75 cm area of skin was excised from the recipient mice in the same manner as described above.

F. A piece of graft skin was applied to the excised skin spot. The grafted skin was clipped into place dermis to dermis using a continual row of autoclips around the graft. Recipient mice were placed under a warming lamp immediately after the procedure.

G. Every 24 hours the five recipient mice (PBS + BALB/c RNA) and five control mice (PBS alone) were visually examined in a coded fashion by the same observer. The percentage of graft area undergoing degeneration was determined based on areas of brown to black coloration or induration. The following results were obtained:

7. As shown by the data table and graph, mice intravenously injected with total cellular RNA (PBS + BALB/c RNA) exhibited substantially lower percentages of graft necrosis than the control mice (PBS alone). For example, at day 17 the percentage of graft necrosis in control mice was over 14 times the percentage of graft necrosis in the recipient mice that received a tolerization treatment of total cellular RNA. Moreover, in the time period evaluated, nearly 50% of the skin grafts of control mice became necrotic, while the extent of necrosis of tolerized mice was less than 5%. These results demonstrate substantial protection from acute transplantation rejection.

8. The specification exemplifies, beginning on page 27 (Example 4) intravenous administration of total cellular RNA from the S1509a tumor cell line. Also exemplified was the introduction of a soluble extract of S1509a cells. Tumor cells are a form of transplanted tissue. Thus, the application exemplifies administering total cellular RNA prior to transplanted tissue. Further, page 21 of the specification teaches that intravenous administration of total cellular RNA elicits immune tolerance to transplant tissue antigens, as further shown by this declaration.

9. The tolerance results observed with tumor cells and skin grafts, based on intravenous administration of RNA, support similar tolerization to autoantigens (of which tumor antigens are a subset) and allergens.

I further declare that statements made in this Declaration are of my own knowledge and are true and that all statements made on information and belief are believed to be true and further these statements were made with the knowledge that willful false

statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 12/10/03

Richard D. Granstein